Analytica Chimica Acta 702 (2011) 205–212

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Method development for the determination of wood preservatives in commercially treated wood using gas chromatography–mass spectrometry

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ARTICLE INFO

Article history:
Received 27 May 2011
Received in revised form 28 June 2011
Accepted 30 June 2011
Available online 12 July 2011

Keywords:
Fungicide
Gas chromatography–mass spectrometry
Wood preservation
Triazole
IPBC
Permethrin

ABSTRACT

Fungicides and insecticides are commonly used preservatives to protect wood products against microbiological degradations. Currently, there is a lack of analytical methods addressing the quantitative determination of a wide range of wood preserving species in wood matrices. In this study, a reliable method was developed for the determination of a mixture of wood preserving agents with differing chemical structures (i.e., properties), including tebuconazole (TAZ), propiconazole (PAZ), 3-iodo-2-propynyl butylcarbamate (IPBC), and permethrin (PER), in pine wood. The analyte recoveries obtained by Soxhlet and multiple-stage sonication extractions were compared. While both extraction methods yielded similar results (80–100%), Soxhlet extraction was found to be less labor-intensive and thus preferred providing also lower RSDs of 1–6%. In comparison to methanol, commonly used as an extraction solvent for triazoles, acetone yielded similar extraction efficiencies for all analytes while reducing the time of sample concentration. The solid phase extraction method for triazoles was adapted to allow for a separation of IPBC and PER from the wood matrix. As opposed to previous studies, three recovery standards were employed, which enabled the correction of individual analyte losses during the sample preparation. The matrix-affected limits of detection (LODs) using gas chromatography with mass spectrometric detection were nearly the same for triazoles 0.07 and 0.21 ng g^{-1} for PAZ and TAZ in sapwood and 0.18 and 0.21 ng g^{-1} in heartwood, respectively. Higher LODs were observed for IPBC and PER: 3.9 and 1.7 ng g^{-1} in sapwood, and 2.0 and 6.0 ng g^{-1} in heartwood, respectively. The recoveries in the wood submitted to commercial sample treatment showed gradient distribution of analytes depending on the penetration of the treatment.

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1. Introduction

Fungicides are widely used preservatives within agriculture, chemical industry, and polymer technology to protect various products against microbiological decay in the outdoor and indoor environment [1–4]. The selection of fungicides to protect a particular product, such as wooden windows, is based on their specific properties (i.e., the ability to control the growth of a particular fungi and their biodegradability) [1,2]. Typically, a mixture of preservatives possessing complementary chemical and biological and thus protective properties is used. To protect windows and doors, a commercially available solution of Woodlife® 111 (Kop-coat, Inc.) is currently employed. Fungicides triazoles, i.e., tebuconazole (TAZ) and propiconazole (PAZ), and 3-iodo-2-propynyl butylcarbamate (IPBC), shown in Fig. 1, are the active components of Woodlife® 111. The treatment efficiency may be further enhanced with the use of pyrethroid insecticides, such as permethrin (PER) (Fig. 1) [1].

Up to date, the quality of a protective treatment and/or the determination of fungicide residue in various matrices (soils/sediments, aqueous samples, foods, pharmaceutical formulations, and wood) was assessed by means of gas (GC) or liquid chromatography (LC). Based on the fungicide or insecticide nature and chromatographic method applied, electron capture, nitrogen phosphorous, UV, mass spectrometric (MS), or ion mobility mass spectrometric (IMS) detectors were employed [2–13]. The majority of these studies focused only on determination of fungicides possessing similar chemical properties, such as triazoles [2,5,6,9]. However, as mentioned above, in wood treating solutions enhanced chemical diversity is favored, thus complicating a single method development for simultaneous analyte determination. The available studies addressing the quantitative determination of a mixture of compounds with differing chemical functionalities, e.g., fungicides and insecticides, are limited [11,13].

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doi:10.1016/j.aca.2011.06.058

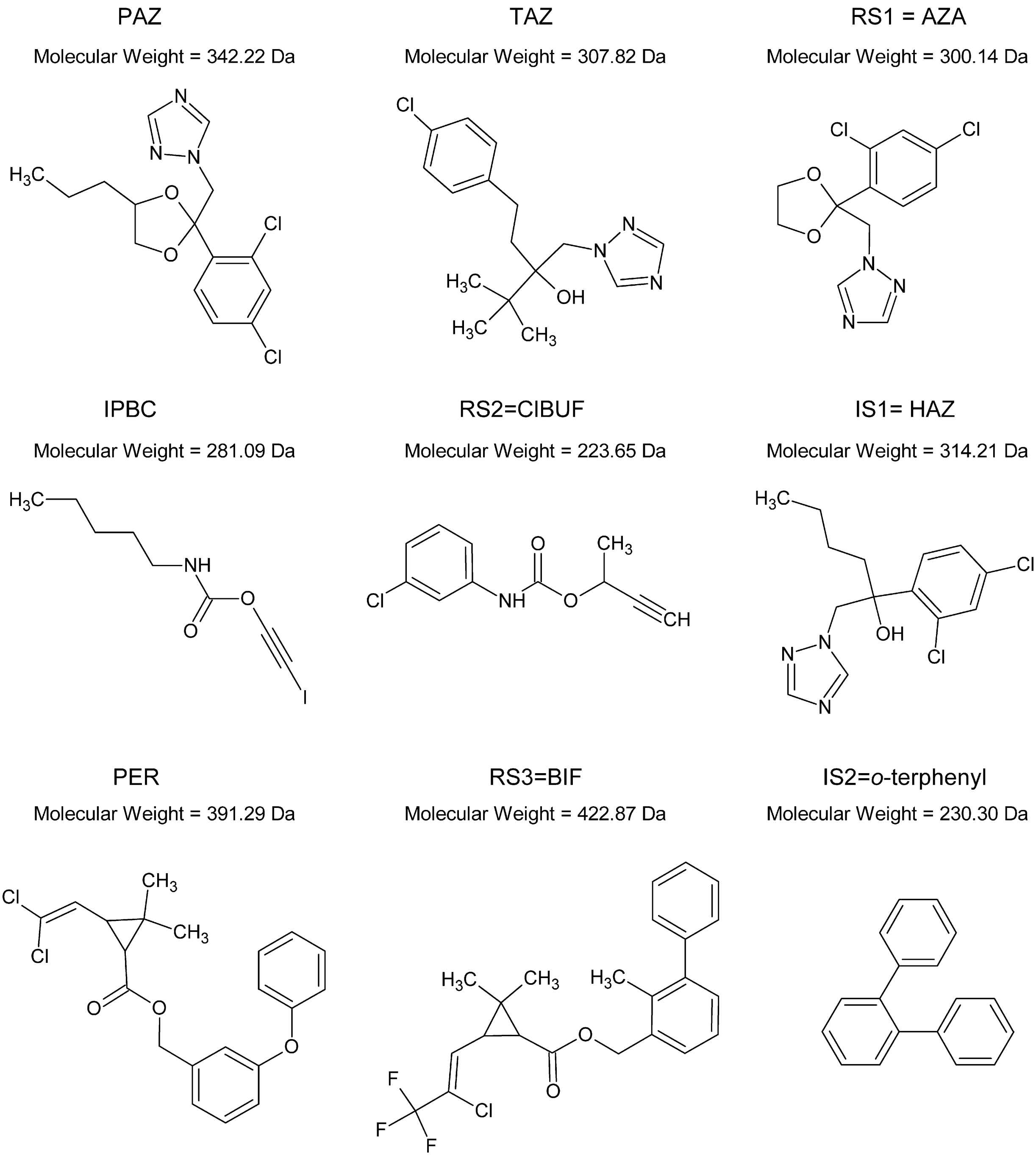
PAZ
Molecular Weight = 342.22 Da
TAZ
Molecular Weight = 307.82 Da
RS1 = AZA
Molecular Weight = 300.14 Da
IPBC
Molecular Weight = 281.09 Da
RS2=ClBUF
Molecular Weight = 223.65 Da
IS1= HAZ
Molecular Weight = 314.21 Da
PER
Molecular Weight = 391.29 Da
RS3=BIF
Molecular Weight = 422.87 Da
IS2=o-terphenyl
Molecular Weight = 230.30 Da

Fig. 1. Structures of wood preservatives, RSs and ISS used.

For triazoles, the reported extraction protocols from wood samples using methanol included a reflux for 1.5–2 h [2,5,9], sonication at 55 °C for 3 h [2,9], or Soxhlet extraction for 5 h [6]. To our best knowledge, there is no systematic study evaluating extraction efficiencies of all preservatives, i.e., triazoles, IPBC and PER, using various solvents and extraction times.

Although numerous interfering wood co-extractives are recovered together with fungicides upon a solvent extraction, the American Wood Preservers Association (AWPA) methods do not require sample purification [5,6]. Depending on the wood matrix, the co-extractives are addressed only by modifications of the HPLC method (i.e., gradient program) [5,6]. However, such protocols are difficult to implement in a routine laboratory setting as the method may need a new modification for each wood type. Furthermore, introducing the impurities directly on an analytical column reduces the column lifetime.

Recently, a solid phase extraction (SPE) method using a mixed cation exchanger (MCX) stationary phase was designed for determination of triazoles in wood enabling a shorter HPLC analysis with a simple isocratic run [2]. Nevertheless, SPE or any other purification method for pyrethroids, i.e., PER, or IPBC in wood extracts has not been developed. In other matrices, such as vegetables [14] and soil [7], pyrethroids were mainly pre-cleaned with Florisil and Silasorb Phenyl SPE, respectively.

As mentioned above, either GC or LC is usually employed for the fungicide analysis. While in most cases the sample preparation involved several steps, no recovery standards (RSs) addressing the analyte losses were employed. Furthermore, in the majority of previous studies an external calibration was typically used [2,3,5,6,8,11,14]. To our best knowledge, only one AWPA method implemented the use of an internal standard (IS) for wood samples, thus addressing possible volume variations [9].

Another approach to determine fungicides in wood matrices involved the use of thermal desorption methods [3,11,13]. Minimal sample pretreatment was required for the pyrolysis GC–MS [11] as well as for the thermal desorption coupled to IMS [3,13] or MS [13]. Even though both methods allowed for fast identification [3], and suggested quantitation of insecticide and fungicides in wood samples, only qualitative results were reported [11,13]. Moreover, for most laboratories such approach is not feasible due to the limited accessibility of pyrolysis or thermal desorption instrumentation.

The goal of this study was to develop a versatile method for the determination of fungicides/insecticide (TAZ, PAZ, IPBC, and PER, i.e., chemicals of strongly differing chemical nature) used for a treatment of pine sapwood and heartwood windows. The method development included several steps, namely (1) evaluation of the extraction method; (2) adaptation of the SPE protocol ensuring the purification of IPBC and a nonpolar insecticide PER; (3) use of appropriate recovery and internal standards; and (4) determination of limits of detection (LODs) and quantification (LOQs) with and without the impact of pine sapwood and heartwood matrix, while using GC–MS. The developed method was applied for the determination of target analytes in wood samples treated with Woodlife® 111 and PER using a standard protocol for the window frame treatments to assess the distribution of the wood treatment.

2. Materials and methods

2.1. Chemicals

Analytical standards: TAZ, PAZ, IPBC, PERM, as well as the following RSs: azaconazole (RS1), chlorobufam (RS2), and bifenthrin (RS3), and ISs: hexaconazole (IS1) and *o*-terphenyl (IS2) of >99% purity, depicted in Fig. 1, were purchased from Sigma–Aldrich (St. Louis, MO, USA). Acetonitrile (ACN), methanol (MeOH), dichloromethane (DCM), and acetone (LCMS or GC grade) were from Fisher Scientific (Pittsburgh, PA, USA). Water was purified using a DirectQ Millipore system (Billerica, MA, USA). Woodlife® 111 (Kop-coat, Inc., Pittsburgh, PA, USA) composed of PAZ, TAZ, and IPBC (each 0.21%), and PER (0.1%) were used for the treatment of wooden blocks at a Marvin Windows and Doors facility (Warroad, MN, USA).

Calibration standards in the concentration range of 0.03–250 μg mL^{-1} of each analyte (TAZ, PAZ, IPBC, and PER) were prepared in DCM using serial dilutions. Ammonium hydroxide (25%, Acros, Morris Plains, NJ, USA) and fuming hydrochloric acid (37%, Fluka) were employed for the preparation of SPE eluents.

2.2. Sample preparation

All samples were prepared in triplicates or quadruplicates. For the initial extraction evaluation, cuts from wood blocks, typically used for window frames (sapwood and heartwood, 1.5 × 1.5 × 1/16, ~2 g) were weighed, spiked with 40.0 μL of the fungicide mixture (2.5 mg mL^{-1} of each PAZ, TAZ, IPBC, and PER in MeOH) and with 4.0 μL of RSs (~10 mg mL^{-1} each in MeOH). The amounts of spiked fungicides were selected to be representative of typical concentrations found in treated wood. The wood was then air dried at ambient temperature for 0.5–1 h and weighed again to verify complete solvent evaporation. The dried wood was ground in a coffee bean grinder (Black & Decker) for 2 min using ~3 s pulses. The insecticide and fungicides were then extracted from the ground wood using several extraction protocols (see Section 2.3).

To examine the effect of moisture content in wood on the insecticide and fungicide recoveries, the wood cuts were spiked with the mix of standards and RSs as described above. Then, water (2 mL) was applied on the entire surface exposed to fungicides. After water penetrated into the wood, samples were air dried until constant weight and processed as described above.

The window treatment and extraction method were evaluated on the samples prepared by a standard protocol performed in a Marvin Doors and Windows facility using a double vacuum technique [15]. Woodlife® 111 and PER were applied to longer pine sapwood blocks (12 × 1.5 × 1.5) simulating the treated windows frames. Three consecutive radial cuts from one side of each wood sample were taken to evaluate the treatment penetration. Wood samples were prepared for the analysis using the same procedure as described above.

2.3. Solid–liquid extraction

To ensure an efficient fungicide extraction, protocols using Soxhlet and sonication extractions with various solvent volumes (40–100 mL) were compared (Table 1). As suggested previously, an extraction solvent makes a significant impact on analyte recoveries [16]. Recommended by the AWPA method, MeOH was selected as an extraction solvent. Due to the wide range of polarities and chemical structures of target analytes, less polar and or more volatile solvents than MeOH, i.e., ACN and acetone, were also examined. Previously, acetone has been successfully applied for wood matrix disruption [16].

Soxhlet extractions were conducted in cellulose thimbles (Whatman Int., Maidstone, UK) with 100 mL of a solvent for 4 h (i.e., a similar protocol to that provided by AWPA [6]). Wood extracts were then evaporated to dryness using a rotary evaporator or under a stream of nitrogen gas.

Sonications were carried out at 55–65 °C using a Branson 2510 ultrasound bath at 42 kHz (Branson Ultrasonics, Danbury, CT, USA) in 40 mL vials, capped with PTFE lined screw tops and sealed with a parafilm. Two sonication protocols (A and B) are described in Table 1. In both protocols, the sonication was followed by several rinses to remove residual insecticide and fungicides from the swollen wood matrix. After each extraction step, the extracts were centrifuged at 5000 rpm for 10 min and let stand for several minutes. The supernatant was collected, fractions combined, and evaporated to dryness as described above. The dried extracts were then redissolved either in deonized water or in MeOH, depending on the selected purification protocol.

2.4. Sample purification

The SPE purification method developed by Miyauchi et al. [2] for triazoles in wood matrix was adapted to ensure the elution of *all* target analytes while removing wood matrix interferences. The method employed Discovery DSC-MCAX (Supelco), a mixed cation exchange and reverse (C8) phase mode (300 mg of the sorbent mass, 3 mL volume) SPE cartridge. SPE was carried out on a 12-port glass vacuum manifold (Alltech, Deerfield, IL, USA) using a combination of gravity and vacuum, not exceeding the elution speed of 2 mL min^{-1}. The SPE method was developed using nontreated wood extracts, which were spiked with target analytes prior to the SPE.

For the analysis of constituents of Woodlife® 111 (TAZ, PAZ, and IPBC), the dried wood extracts were dissolved in 0.50 mL deionized water followed by MCAX SPE 1 protocol (described in Table 2). IS1 (4.0 μL, 10 mg mL^{-1} in MeOH) was added to fractions I and II, which were then evaporated to dryness, re-dissolved in 0.90 mL of DCM, and analyzed using GC–MS. For the purification of wood extracts spiked with constituents of Woodlife® 111 and PER, MCAX

Table 1
The effect of the extraction method and solvent on PAZ, TAZ, IPBC, and PER (2.5 mg mL^{-1} of each in MeOH) recoveries from pine sapwood and heartwood. Data are expressed as mean and SD ($n = 3$ or 4).

Method	Solvent	Wood	Amount (mL)	Time	Protocol	TAZ	PAZ	IPBC	PER	TAZ	PAZ	IPBC	PER
Sonication A[a]	MeOH	Sap	40	8 h	20 mL + 2× 10 mL rinses	77.8 ± 0.4	69 ± 3	58 ± 4	–	–	–	–	–
Sonication B[b]	MeOH	Sap	80	3× 1 h	3× 20 mL + 2× 10 mL rinses	86 ± 8	85 ± 8	78 ± 3	78 ± 3	96 ± 3	95 ± 2	88 ± 9	101 ± 6
Sonication B[b]	MeOH	Heart	80	3× 1 h	3× 20 mL + 2× 10 mL rinses	78 ± 3	77 ± 3	65 ± 2	51 ± 3	99 ± 3	99 ± 3	97 ± 6	77 ± 8
Sonication B[b]	Acetone	Sap	80	3× 1 h	3× 20 mL + 2× 10 mL rinses	86 ± 7	86 ± 6	74 ± 11	79 ± 10	96 ± 1	96 ± 1	90 ± 3	97 ± 1
Sonication B[b]	Acetone	Heart	80	3× 1 h	3× 20 mL + 2× 10 mL rinses	72 ± 3	73 ± 3	71 ± 5	54 ± 4	95 ± 1	96 ± 1	96 ± 3	72 ± 3
Soxhlet[b]	MeOH	Sap	100	4 h		85.7 ± 0.9	76.1 ± 0.4	75 ± 8	–	–	–	–	–
Soxhlet[b]	Acetone	Sap	100	4 h		91 ± 3	83 ± 5	100 ± 5	100 ± 5	103 ± 4	94 ± 4	104 ± 2	102 ± 2
Soxhlet[b]	Acetone	H_2O treated	100	4 h		87 ± 51	68 ± 4	92 ± 5	86 ± 5	103 ± 4	81 ± 4	99 ± 6	103 ± 3

[a] Application of SPE 1.

[b] Application of SPE 2.

Table 2
SPE protocols employed for the wood extract purification. MCAX SPE 1 protocol was used for a mix of PAZ, TAZ, and IPBC. In MCAX SPE 2 protocol, a mixture of PAZ, TAZ, IPBC, and PER were purified.

MCAX SPE 1 protocol	
Conditioning	3 mL MeOH
	3 mL 1 mM HCl
	Sample load (0.5 mL in water) + 3 mL of 30% MeOH in water
Fraction I	3 mL of 100% MeOH
Fraction II	5 mL of 2% NH_4OH in MeOH
MCAX SPE 2 protocol	
Conditioning	3 mL MeOH
	3 mL 1 mM HCl
	2 mL MeOH
Fraction A	Sample load (0.5 mL in MeOH) + 5 mL of 100% MeOH
Fraction B	5 mL of 2% NH_4OH in MeOH

SPE 2 protocol was used (Table 2). In this protocol, the dried wood extracts were dissolved in 0.50 mL of MeOH and then loaded on a preconditioned column. ISs (4.0 μL of each, 10 mg mL^{-1} in MeOH) were added to each fraction, evaporated to dryness, re-dissolved in 0.90 mL DCM, and analyzed on GC–MS. Even though it was previously reported that IPBC is thermally unstable [8], we did not experience any difficulties when analyzing IPBC on GC–MS. However, MeOH was not a suitable solvent for a direct GC–MS analysis; it caused distortion (splitting) of the IPBC chromatographic peak.

2.5. *Instrumentation*

Gas chromatographic analyses were performed using a 5890 Series II Plus GC coupled to a 5972 MS (Hewlett-Packard, Santa Clara, CA). Separations were carried out using a 30 m-long DB-5MS column with 0.25 mm internal diameter and 0.25 μm film thickness (J&W Scientific, Rancho Cordova, CA, USA) at a constant helium flow rate of 0.90 mL min^{-1}. Samples (1.0 μL) were injected in a splitless (0.5 min) mode at 250 °C. The GC temperature program started at 40 °C, held for 1 min, then heated to 300 °C with 10 °C min^{-1} temperature gradient, and held for 3 min; a total run time was 30 min. The transfer line was set to 280 °C. The MS data were acquired in the full scan mass range of 43–500 m/z using the electron ionization (70 eV).

2.6. *Data processing*

The analytes were identified based on their retention times and mass spectra matching those of individual standards. Table 3 lists the quantifying (with the highest signal to noise ratio) and qualifying ions for each analyte of interest as well as for RSs and ISs. The internal standard method was used to quantify the analytes. Two stereoisomers of PAZ and PER were separated in the GC chromatograms (Figs. 2 and 3), and their peak areas were summed for the quantitation.

Both instrumental and matrix-affected LODs were calculated from the calibration curves generated using a least square linear

Table 3
GC–MS quantifying and qualifying ions used for data processing.

	Quantifying ion (m/z)	Qualifying ions (m/z)
PAZ	173	182, 57, 55
TAZ	125	259, 175, 69
IPBC	165	250, 70, 83
PER	183	163, 77, 165
RS1:AZA	217	173, 219, 175
RS2: ClBUF	53	127, 164, 223
RS3: BIF	181	165, 141
IS1: HAZ	83	214, 82, 216
IS2: *o*-terphenyl	230	229, 215, 202

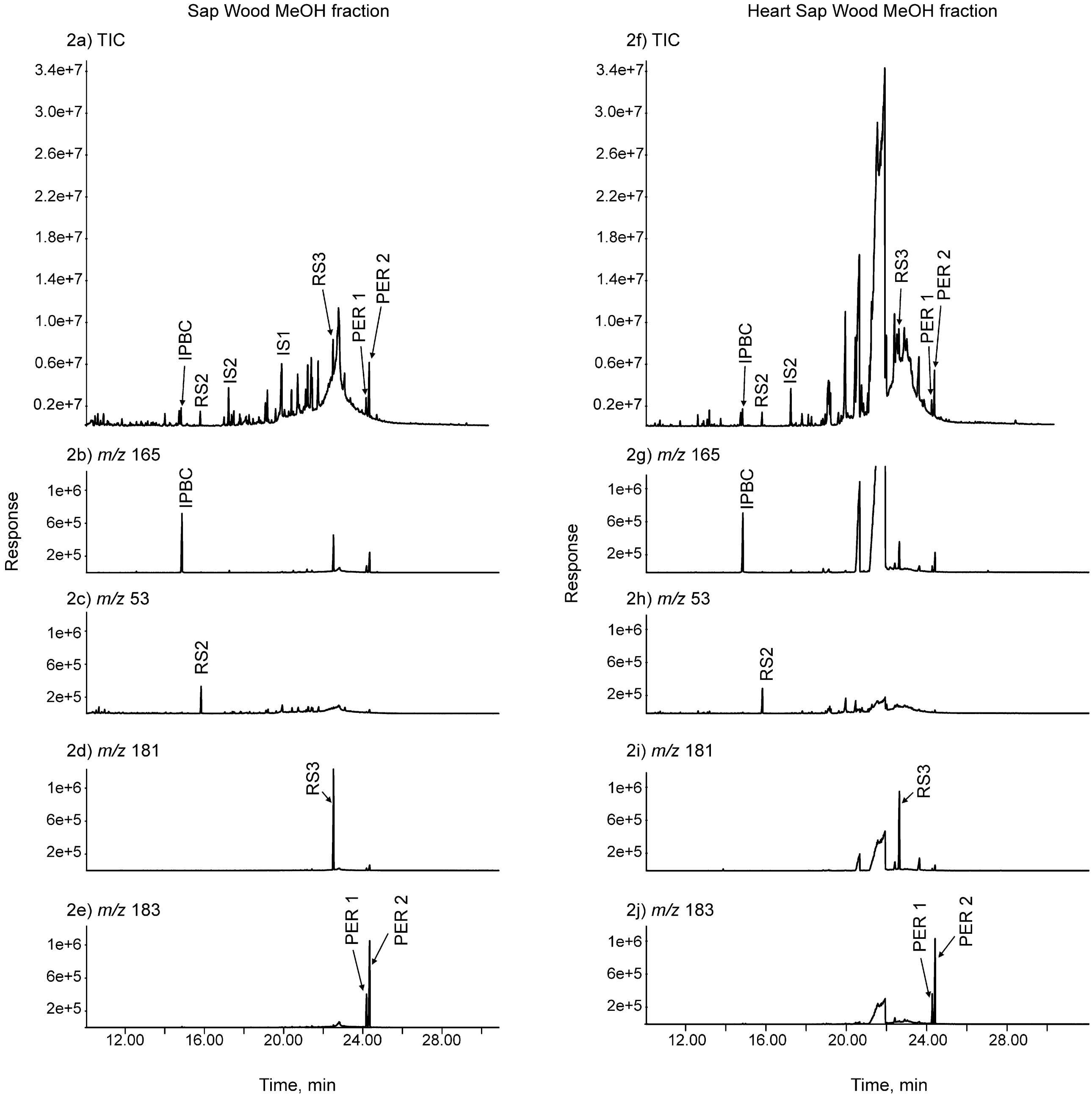

Fig. 2. TIC and extracted ion GC–MS chromatograms of methanol fractions eluting IPBC and PER from pine sapwood (a–e) and heartwood (f–j) samples.

regression. The following equations were used: LOD = 3.3*s_y/k and LOQ = 10*s_y/k [17], where s_y is a standard error of the predicted y-value for each x-value and k is a slope of a calibration curve. For the LOD calculations, only the points within one order of magnitude of the LOD were used. The instrumental LODs were expressed per injected volume. To determine the influence of a matrix on LODs, sapwood and heartwood extracts of known volume were spiked with PAZ and TAZ, and IPBC and PER in the concentration range of one order of magnitude of LOD.

3. Results and discussion

3.1. Extraction recoveries

Four parameters, namely, the extraction method, solvent, solvent volume, and extraction time were taken into consideration when evaluating the extraction efficiency (Table 1). Initial work started with MeOH, as recommended by the AWPA methods [5,6], attempting to reduce time and instrumental requirements for the extraction of individual components of Woodlife® 111 (i.e., TAZ, PAZ, and IPBC). The results shown in Table 1 indicate that the sonication extraction efficiency depended on the analyte partitioning into a sufficient solvent volume. Even though in sonication protocol A the extraction time was the longest, taking 8 h, it resulted in the lowest extraction recoveries from sapwood, 78, 69, and 58% of TAZ, PAZ, and IPBC, respectively. By contrast, sonication protocol B employing a shorter sonication time (4 h) and larger solvent volume (80 mL vs. 40 mL in the protocol A) resulted in a 10–20% increase in the extraction efficiency (Table 1). Similar recoveries to those in the sonication protocol B were obtained with a 4 h Soxhlet extraction using 100 mL of MeOH. However, sonication protocol B gave one of the largest standard deviations (SDs) possibly caused by multiple-step sample handling.

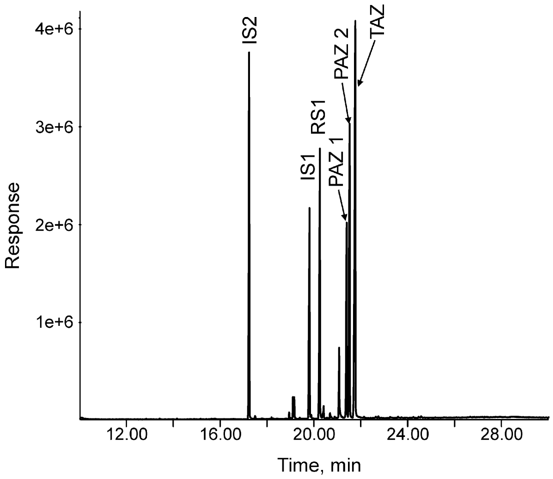

Fig. 3. TIC GC–MS chromatograms of the ammonia hydroxide fraction eluting triazoles from a heartwood sample.

Since heartwood, located in the center of a tree, grows longer than sapwood, it becomes more compact. Thus, the matrix disruption was not expected to be as efficient as in the case of sapwood. Nevertheless, with the exception of PER, the fungicide extraction recoveries were comparable to those achieved by sonication protocol B for sapwood (Table 1).

Besides MeOH, extraction efficiency using acetone was also examined. Acetone showed comparable extraction recoveries for all fungicides from sapwood and heartwood with half the sample evaporation time. It was also verified, that both evaporation techniques (using N_2 or rotary evaporator) provided ca. 95 ± 5% recoveries. Moreover, when the use of acetone was combined with the Soxhlet extraction protocol, increased recoveries ~100% for TAZ, IPBC, and PER and 81% for PAZ were observed (Table 1) Thus, the *Soxhlet extraction* method with *acetone* seemed to be the most efficient in terms of the extraction recovery, extraction time, and sample handling. The amount of matrix interferences extracted along with the analytes of interest was comparable when employing either solvent, i.e., MeOH and acetone. When using ACN the analyte recoveries were much lower than those when using acetone or MeOH (data not shown). Moreover, elution of some white interfering matrix was observed when ACN was used as the extraction solvent.

As stated above, RSs were not previously employed even though a multiple-step sample preparation is necessary prior to the analysis. In this study, three RSs were implemented based on their chemical structures in the following manner: RS1 for the triazoles, RS2 for IPBC, and RS3 for PER. The importance of using RSs when extracting the analytes of interest can be seen from the data in Table 1. The correction to individual RSs in terms of obtaining the accurate values of analyte recoveries lead to near-100% recoveries for TAZ and PAZ, to 88–104% for IPBC, and to 72–102% for PER. In addition, the RS corrections lowered the SDs.

Table 1 also shows the effect of moisture of the treated wood on extraction efficiency. Recoveries of TAZ and IPBC were not affected; however, a ~10–15% loss of PER and PAZ was observed. The apparent loss of PER was associated with the sample preparation steps and the application of RS3 was able to account for it.

3.2. Purification

The evaluation of sample cleanup methods resulted in two protocols provided in Table 2. For the purification of extracts obtained by a treatment with constituents of Woodlife® 111 (TAZ, PAZ, and IPBC), the MCAX SPE1 method was optimal. IPBC eluted in the second methanol fraction, and TAZ and PAZ in the ammonium hydroxide fraction. The benefit of this protocol was the elimination of polar interferences with a washing step using 3 mL of 30% MeOH in water. Initially, we acidified samples prior to the purification as recommended by the manufacturer for the use of DSC–MCAX SPE cartridges. However, this step was later eliminated as IPBC losses were observed, possibly due to its acidic hydrolysis [18].

The MCAX SPE1 protocol was, however, not applicable to PER due to its limited solubility in water [14] causing the coagulation of wood extracts prior to the SPE. To allow the purification of all active ingredients, MCAX SPE 2 protocol (Table 2) was employed. In this protocol, 2 mL of MeOH were loaded on the SPE column prior to the sample load to avoid sample precipitation. The recoveries and elution obtained for MCAX SPE2 protocol (Table 2) were similar to the MCAX SPE1 protocol, IPBC and PER eluted in the methanol fraction while triazoles eluted in the ammonium hydroxide fraction. Since the first fraction of this protocol was not prewashed, we attempted to purify it further using a second reverse (C18) phase SPE. However, this extra purification step was unsuccessful. Therefore, when analyzing heartwood samples, a liner with glass wool had to be changed after each sequence or 100 injections. In case of the sapwood samples, the liner lifetime could be extended to several sequences.

The effectiveness of the purification method is demonstrated in Figs. 2 and 3 for both types of wood. It is apparent that the purification method was not as effective for a more complex heartwood matrix in TIC mode (Fig. 2f). However, when individual ions were extracted (Fig. 2g–j), no interfering species were observed. The ammonium hydroxide fraction with triazoles showed minimum analyte interference as depicted in Fig. 3.

3.3. Limits of detection

A good linearity was achieved for all analytes in the range from LOQs to 250 $\mu g\,mL^{-1}$, which is a wider range than that used in the AWPA methods (10–200 $\mu g\,mL^{-1}$) [5,9]. The slope, s_y, regression

Table 4
Calibration parameters obtained from a least square regression in a concentration range 0.03–250 $\mu g\,mL^{-1}$ per analyte using at least 10 dilutions and injection volume of 1 μL. The instrumental and matrix-affected LODs/LOQs were obtained as described in the experimental section. LODs and LOQs were then expressed per injected volume and wood weight.

	Instrumental parameters					Matrix-affected parameters			
	Slope	s_y	R^2	LOD (ng)	LOQ (ng)	Sapwood[a]		Heartwood[b]	
						LOD (ng g^{-1})	LOQ (ng g^{-1})	LOD (ng g^{-1})	LOQ (ng g^{-1})
TAZ	0.018	0.029	0.9996	0.03	0.09	0.07	0.22	0.21	0.64
PAZ	0.014	0.023	0.9996	0.05	0.15	0.22	0.66	0.18	0.54
IPBC	0.018	0.015	0.9999	0.40	1.2	3.9	12	2.0	6.0
PER	0.032	0.033	0.9998	0.07	0.20	1.7	5.2	6.7	20

[a] Sap wood weight of 0.43711 g.

[b] Heart sap wood weight of 0.41481 g.

Table 5
The distribution of the preservatives in sapwood following the treatment with a double vacuum technique analyzed at two allotted times within a 10-day period (inter-day repeatability) for selected samples. The results are presented as the averages, standard deviations and relative standard deviations of three independent treatments. The penetration is demonstrated by determination in three consecutive wood cuts.

Cut No.	TAZ		PAZ		IPBC		PER	
	Average (μg g^{-1})	RSD (%)	Average (μg g^{-1})	RSD (%)	Average (μg g^{-1})	RSD (%)	Average (μg g^{-1})	RSD (%)
1	702	6	807	4	681	12	336	4
2	408	18	500	16	290	2	251	16
3	321	5	391	4	207	4	193	2
Inter day repeatability								
1	736	3	823	2	714	10	349	2
2	478	8	565	9	335	8	267	15
3	343	2	410	2	235	4	203	3

coefficients R^2, instrumental and matrix-affected LODs and LOQs for each analyte of interest are listed in Table 4. IPBC exhibited the instrumental LOD being one order of magnitude higher than those of the other compounds used. Furthermore, the developed method allowed for two orders of magnitude lower detection of PER than those obtained by GC×GC-μECD [19]. PAZ, TAZ, and PER instrumental LODs were observed to be two orders of magnitude higher than those obtained by much more selective LC–MS/MS method [20,21]. However, such technique is typically more prone to ion suppression due to matrix interferences and may not be available in routing laboratory. If necessary, a higher sensitivity could be attained if analyzed by GC–MS in the selected ion monitoring mode.

The wood matrix interferences rendered impossible the IS1 detection in the first MeOH fraction. In this case, IS2 was used for quantifiction. For the other fractions, both ISs could be used interchangeably. While LODs for triazoles in sapwood and heartwood were only slightly affected by the wood matrix; those of IPBC and PER were significantly influenced by the matrices (Table 4). This is in an agreement with triazoles being present in the purified ammonium hydroxide fraction whereas, the methanol fraction contained besides PER and IPBC also other matrix interferences. Fungicide/insecticide LODs in the wood extracts could not be compared to those found in the literature as, to our best knowledge, no such results were previously reported.

3.4. Method application to the commercially treated wood

The distribution of the preservative treatment was examined using double-vacuum wood blocks treated at a Marvin Windows and Doors facility with Woodlife® 111 and PER. Three consecutive cuts from each wood block were prepared as described in the experimental section. The concentration of individual fungicides and insecticide found in the three wood cuts are reported in Table 5. The values in each cut are expressed as the averages from the three treated wood blocks. The highest analyte concentrations were found in the first layer. The amount ratio of PER to other compounds was in the first layer in accordance with that of PER to Woodlife® 111 applied to the wood samples. The amount of preservatives decreased by nearly 30% in each consecutive cut. Additional fourth and fifth cuts showed the concentrations similar to those in the third cut (data not shown). The intraday repeatability of the GC–MS analysis was investigated analyzing each wood sample extract in triplicate. A good intraday repeatability, with the relative standard deviations (RSDs) below 3.7%, was achieved for all analytes for both the first and third cuts of individual wood blocks.

The inter-day repeatability was demonstrated by another GC–MS analysis performed in 10 days (Table 5). The RSDs within each cut ranged between 2% and 18% (Table 5). Higher RSDs may be explained by the irregularities in the wood matrix affecting the penetration of fungicides into the wood and, potentially, by the post-treatment wood handling and long-term (4 months) storage.

This high variation was in contrast to the maximum of 5% RSD for wood spiked with defined concentrations of fungicides (Table 1). While developing method for the determination of wood preservatives, their amounts were clearly defined. However, in the commercially treated wood the exact concentration of active ingredients is not known as it depends on parameters described above. In this study, the accuracy of the developed method was determined evaluating the extraction recoveries from the wood samples spiked with the exact concentration of the analytes of interest.

4. Conclusions

The developed method allowed for the determination of a mixture of fungicides and an insecticide possessing various chemical structures and polarities in two different wood matrices. The sample preparation protocol involved grinding of wood samples, Soxhlet extraction with acetone, purification of target analytes with MCAX SPE, followed by the GC–MS analysis. In this work, the extraction and treatment methods were independently evaluated for the first time. High precision, within 5% RSD, of the suggested analytical protocol (excluding the treatment) was achieved. The importance of RSs was demonstrated attaining near-100% analyte recoveries. The low LODs of this method are suitable for the trace analysis of TAZ, PAZ, IPBC, and PER. The method was applied to several wood blocks treated with Woodlife® 111 and PER using double vacuum (a common commercial procedure) showing a significant variation in the analyte distribution across the wood. This uneven distribution should be considered when developing commercial wood treatment methods.

Acknowledgments

The authors would like to acknowledge Marvin Windows and Doors Company for their financial support and Dr. E. Kozliak (UND) for his help editing the manuscript.

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